

Cleave tags

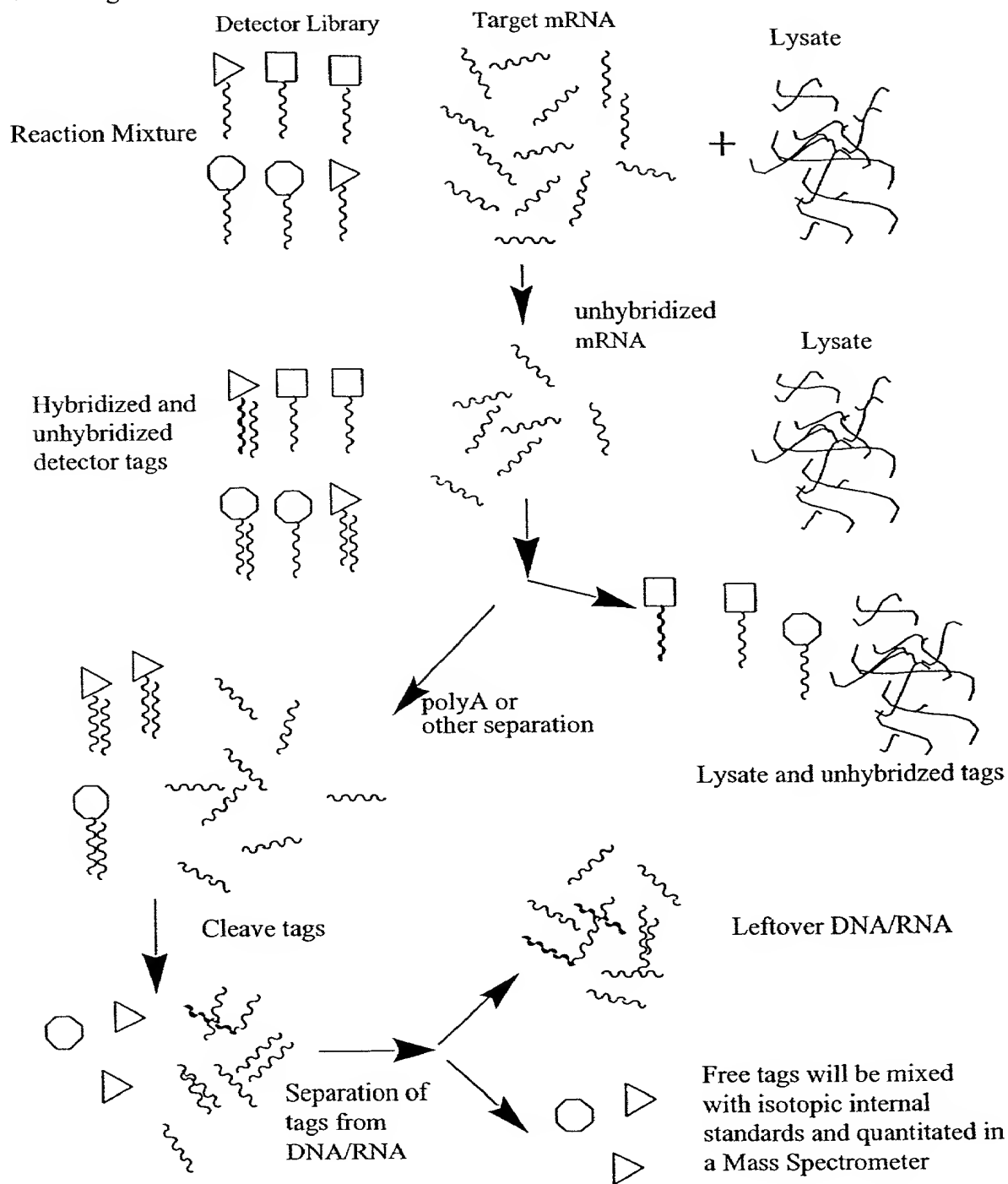


FIGURE 2

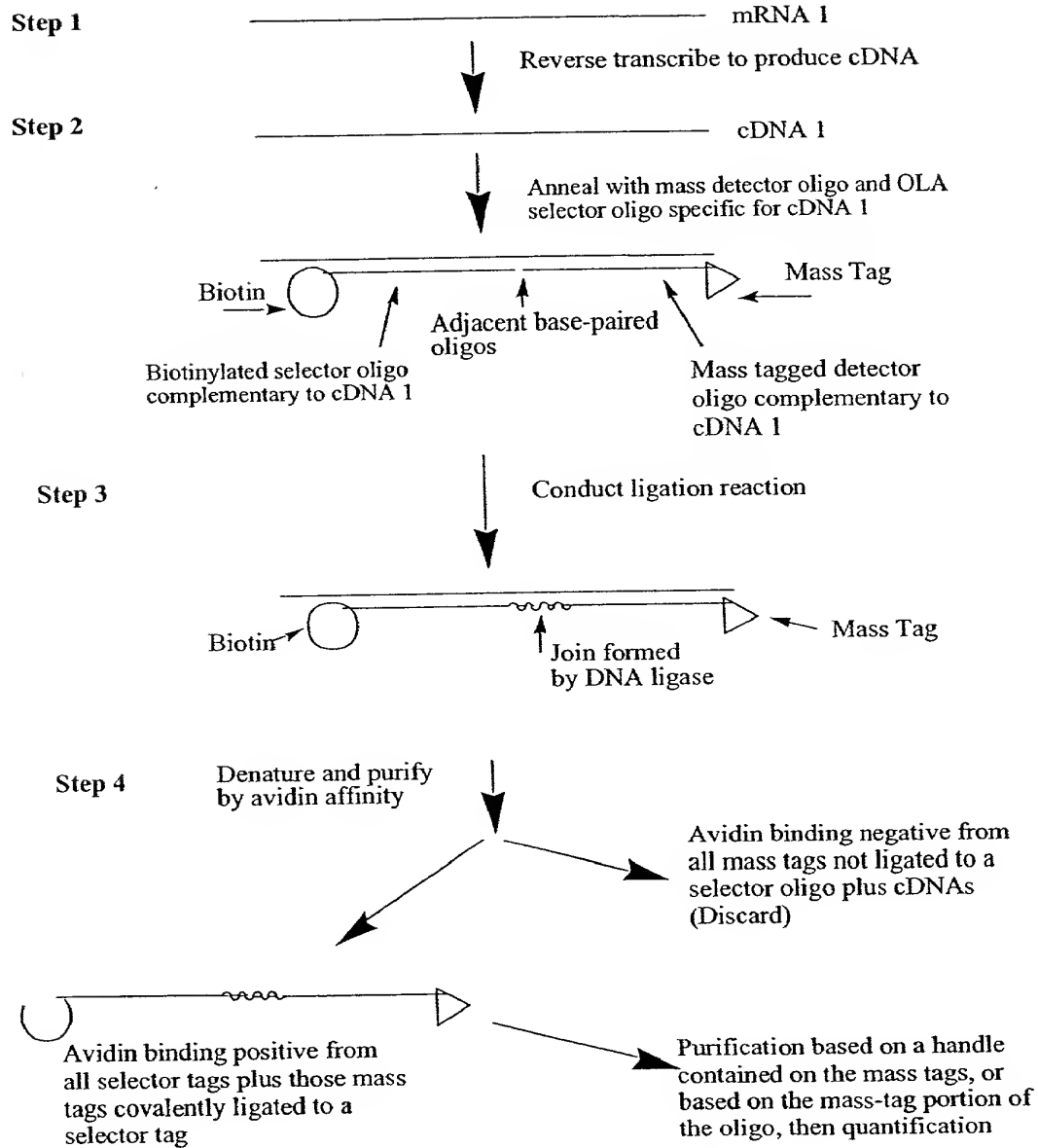


FIGURE 3

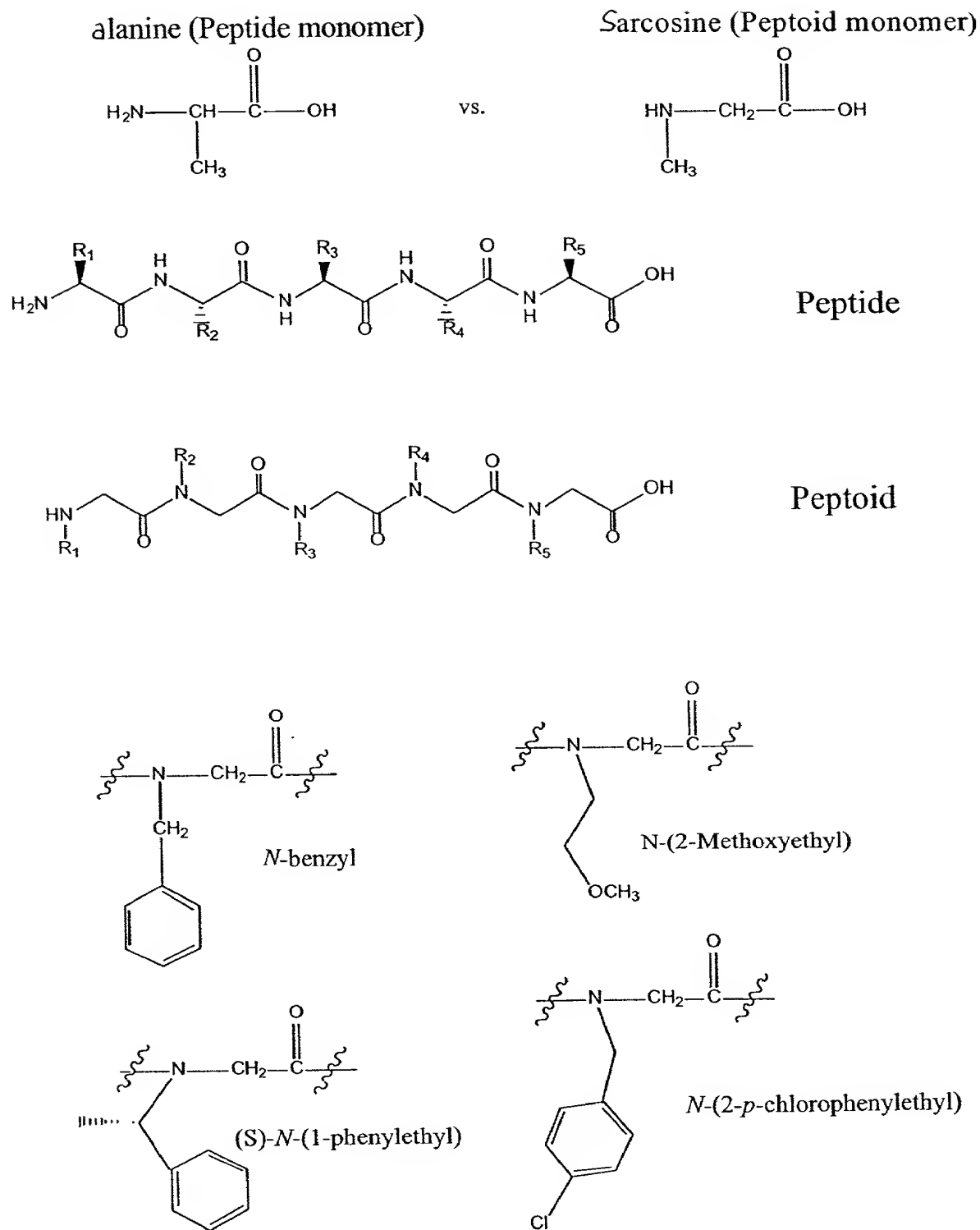


FIGURE 4

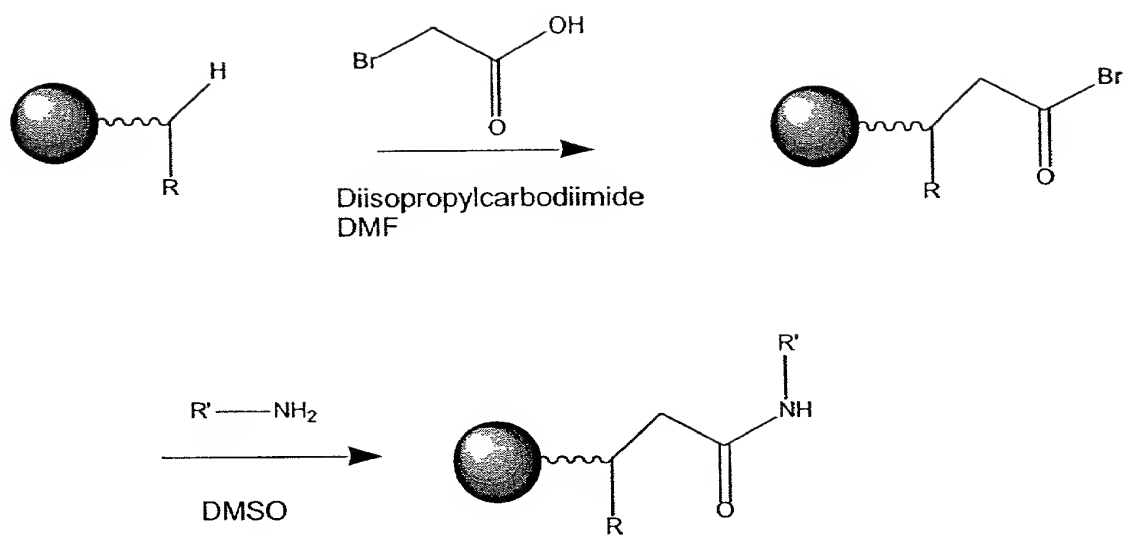
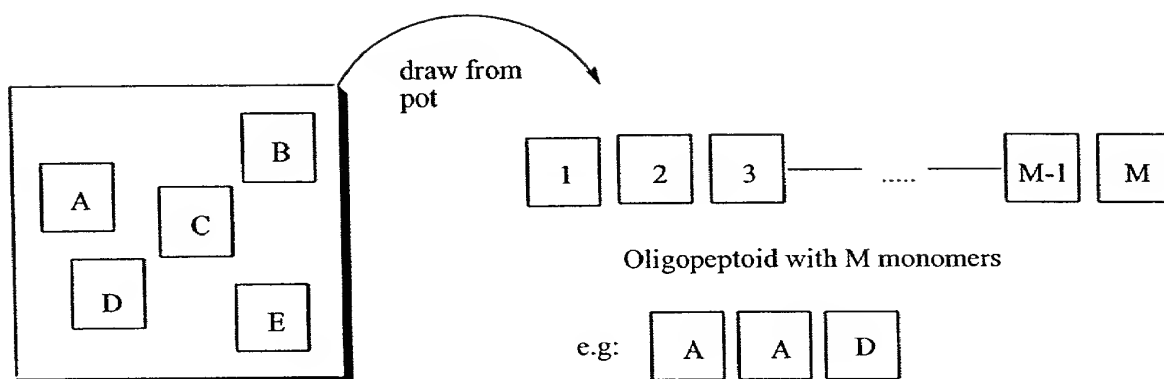


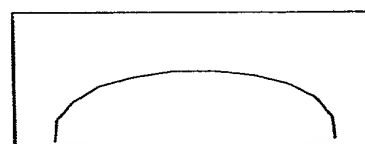
FIGURE 5



$L = \# \text{ of different peptoids with a unique combination of monomers}$

$$L = \binom{M+N-1}{N}$$

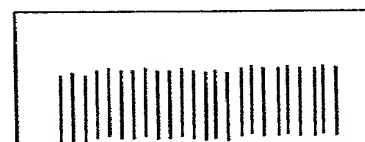
Example: 10 monomers, hexamer peptoids, yields 5005 combinations, or 8007 if pentamers and fewer are used too.



Mostly uniform distribution of molecular weights

Process library by removing coincidental combinations, when two unique combinations have the same mass

Also enforce other requirements, such as a minimum of X Daltons between species, or structural requirements such as at least two charged bases.

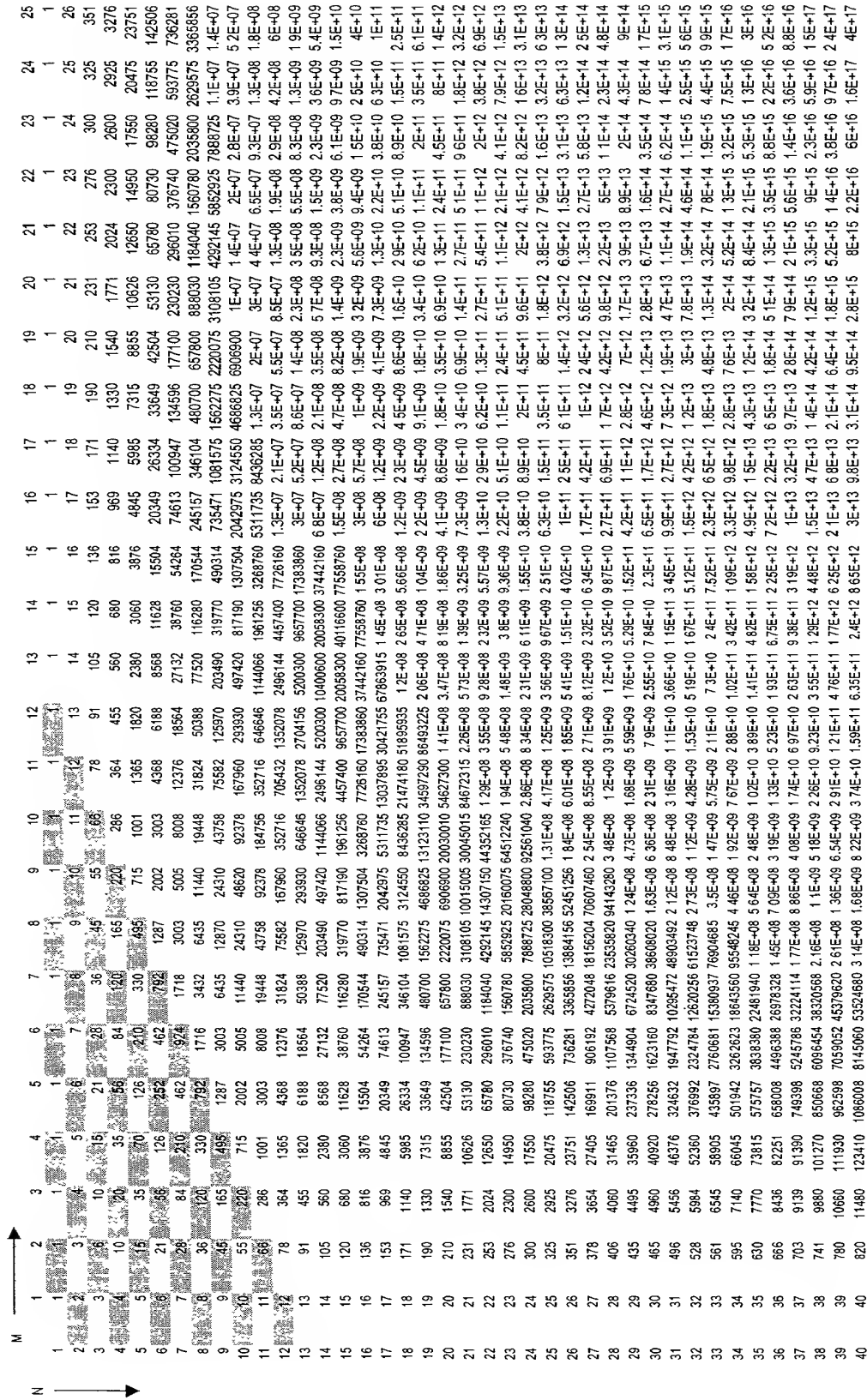


Desirable distribution of molecular weights

Assign oligo probes to tags and log, output library structure to peptoid synthesizer

FIGURE 6

Figure 7



N=length of polypeptide
N=# of monomers of unique mass

	1	2	3	4	5	6	7	8	9	10	11	12
1	1	2	3	4	5	6	7	8	9	10	11	12
1	1	2	3	4	5	1	6	7	8	9	1	10
2	2	5	9	14	20	27	35	44	54	65	77	90
3	3	9	19	34	55	83	119	164	219	285	363	454
4	4	14	34	69	125	209	329	494	714	1000	1364	1819
5	5	20	55	125	251	461	791	1286	2001	3002	4367	6187
6	6	27	83	209	461	923	1715	3002	5004	8007	12375	18563
7	7	35	119	329	791	1715	3431	6434	11439	19447	31823	50387
8	8	44	164	494	1286	3002	6434	12869	24309	43757	75581	125969
9	9	54	219	714	2001	5004	11439	24309	48619	92377	167959	293929
10	10	65	285	1000	3002	8007	19447	43757	92377	184755	352715	646645
11	11	77	363	1364	4367	12375	31823	75581	167959	352715	705431	1352077
12	12	90	454	1819	6187	18563	50387	125969	293929	646645	1352077	2704155
13	13	104	559	2379	8567	27131	77519	203489	497419	1144065	2496143	5200299
14	14	119	679	3059	11627	38759	116279	319769	817189	1961255	4457399	9657699
15	15	135	815	3875	15503	54263	170543	490313	1307503	3268759	7726159	17383859
16	16	152	968	4844	20348	74612	245156	735470	2042974	5311734	13037894	30421754
17	17	170	1139	5984	26333	100946	346103	1081574	3124549	8436284	21474179	51895934
18	18	189	1329	7314	33648	134595	480699	1562274	4686824	13123109	34597289	86493224
19	19	209	1539	8854	42503	177099	657799	2220074	6906899	20030009	54627299	141120524
20	20	230	1770	10625	53129	230229	888029	3108104	10015004	30045014	84672314	225792839
21	21	252	2023	12649	65779	296009	1184039	4921444	14307149	44352164	129024479	354817319
22	22	275	2299	14949	80729	376739	1560779	5852924	20160074	64512239	193536719	548354039
23	23	299	2599	17549	98279	475019	2035799	7888724	28048799	92561039	286097759	834451799
24	24	324	2924	24754	118754	593774	2629574	10518299	38567099	131128139	471225899	1251677699
25	25	350	3275	23750	142505	736280	3365855	13884155	52451255	183579395	600805295	1852482995
26	26	377	3653	27404	169910	906191	4272047	18156203	70607459	254186855	854992151	2707475147
27	27	405	4059	31464	201375	1107567	5379615	23535819	94143279	348330135	1203322287	3910797435
28	28	434	4494	35959	237335	1344903	6724519	30260339	124403619	472733755	1676056043	5586853479
29	29	464	4959	40919	278255	1623159	8347679	38608019	163011639	635745395	2311801439	7898654919
30	30	495	5455	46375	324631	1947791	10295471	48903491	211915131	847660527	315946	

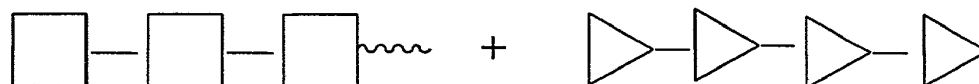
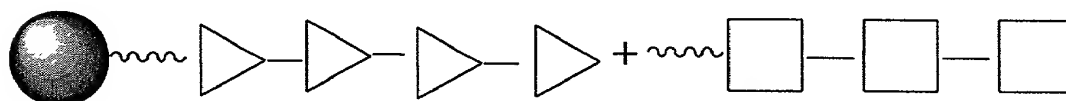
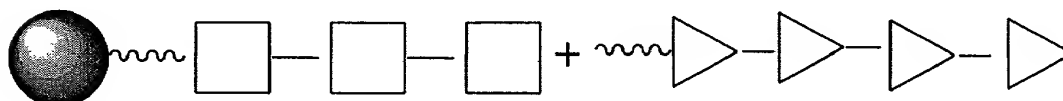
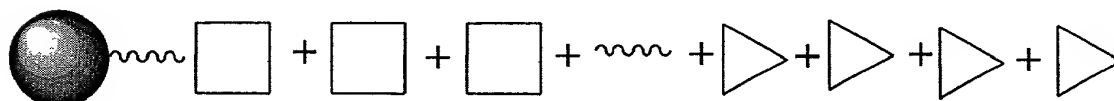
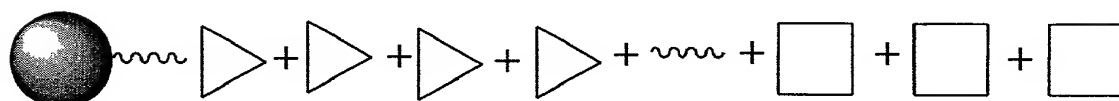
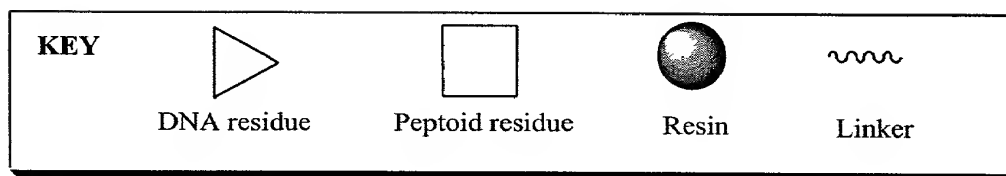
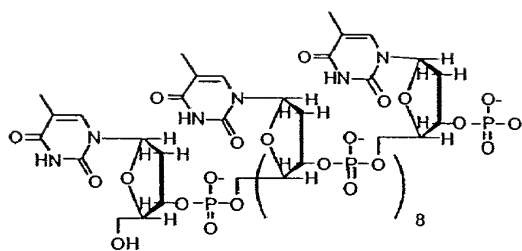
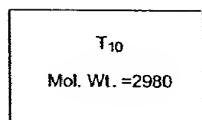
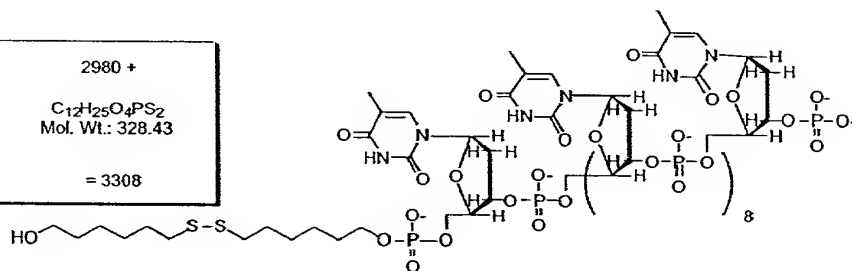
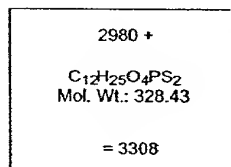


FIGURE 9

N1



N2



N3

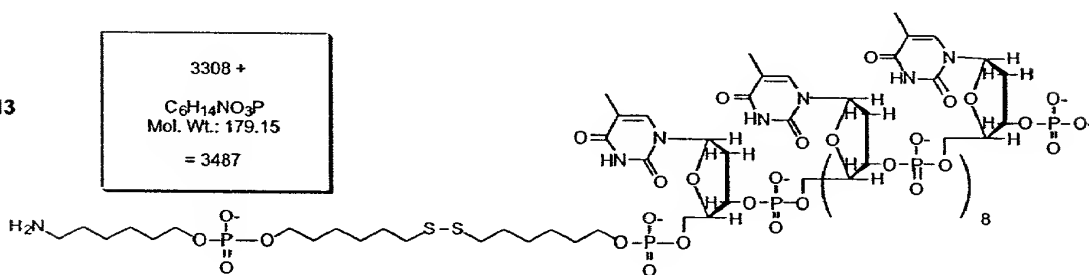
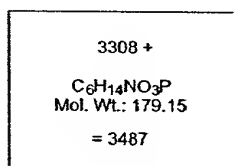


FIGURE 10

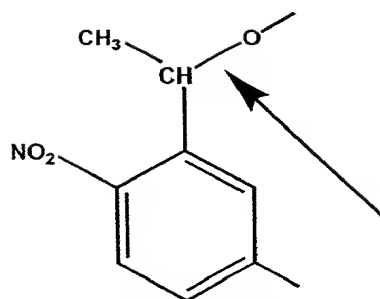


FIGURE 11A

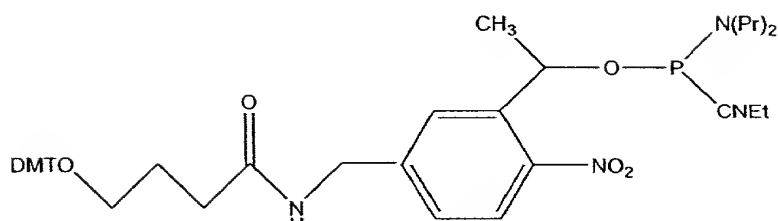


FIGURE 11B

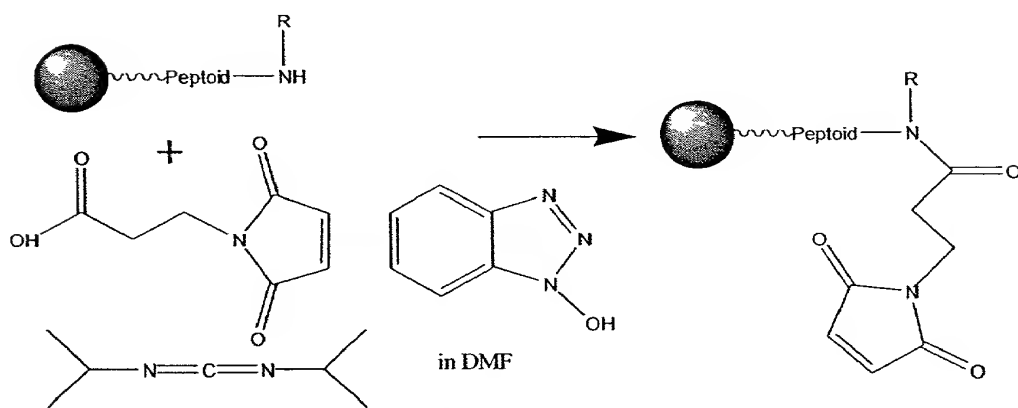


FIGURE 11C

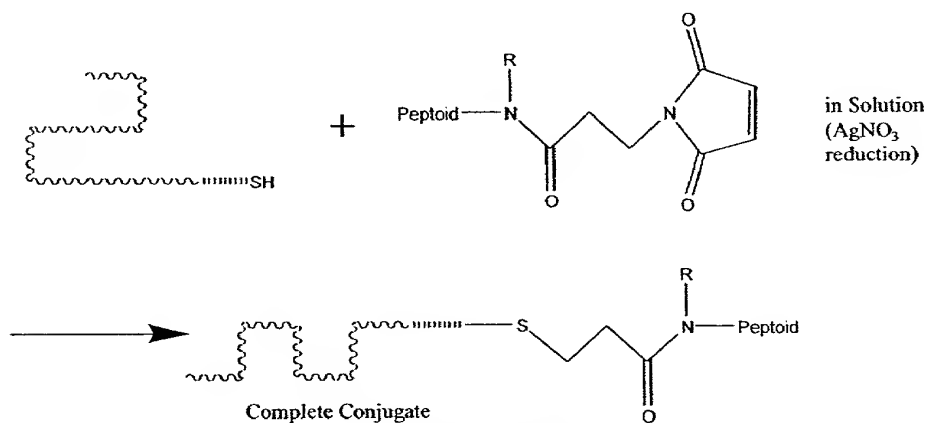


FIGURE 11D

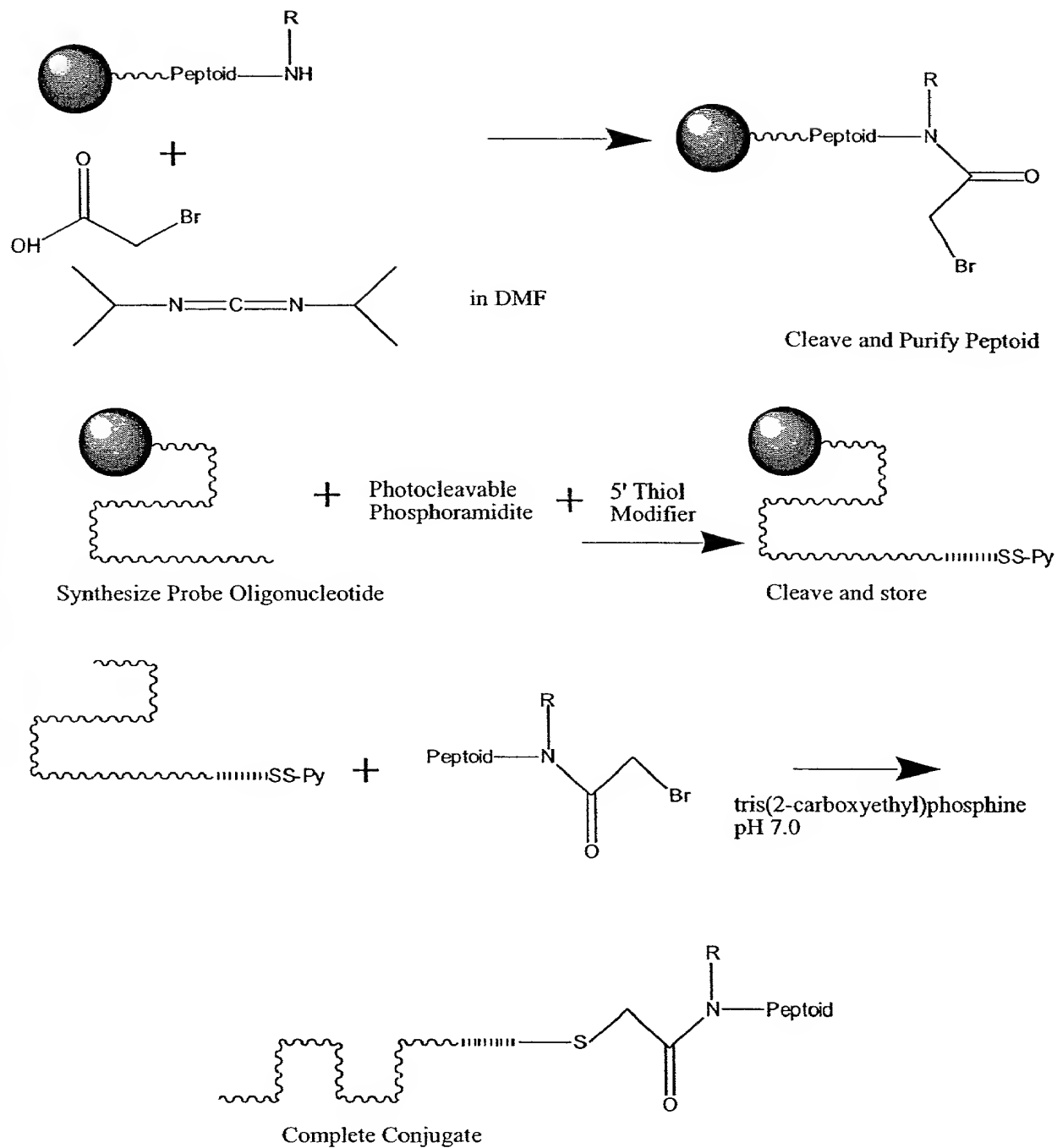
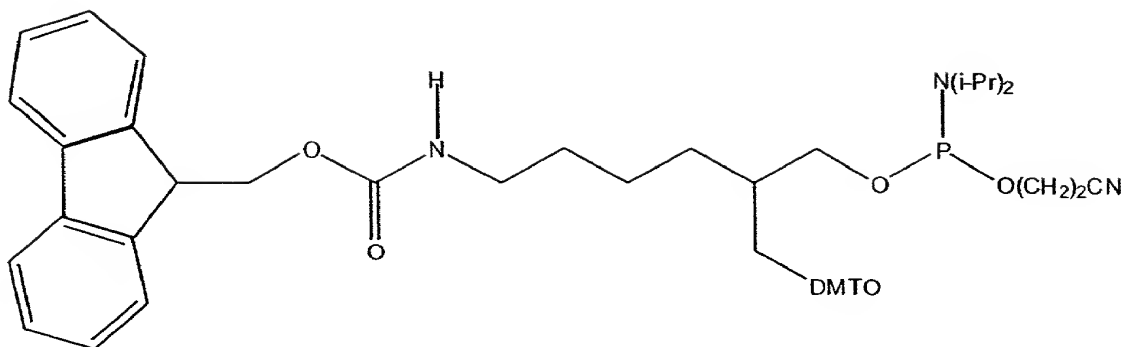


FIGURE 11E



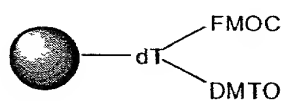
Clontech "Uni-Link AminoModifier" Branched Phosphoramidite

Method

1. Obtain Oligonucleotide Resin with dT base



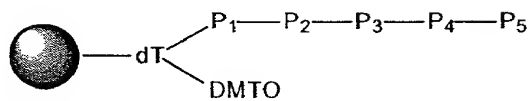
2. Add Branched Phosphoramidite



3. Transfer to Peptoid Synthesizer

4. Deprotect Fmoc

5. Add Peptoid



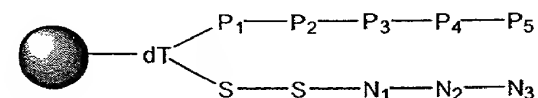
6. Protect Terminus

7. Return to ODN Synthesizer

8. Deprotect DMTO

9. Add cleavable units (Disulfide or Photocleavable)

10. Synthesize ODN



11. Deprotect and Cleave Completed Unit

FIGURE 12

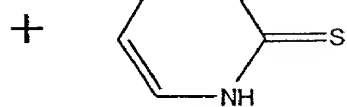
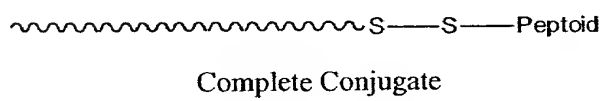
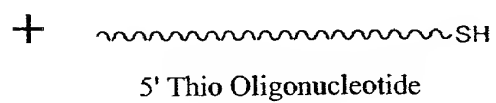
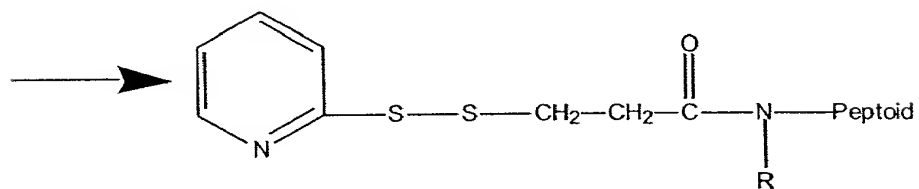
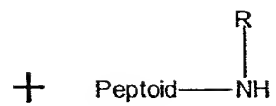
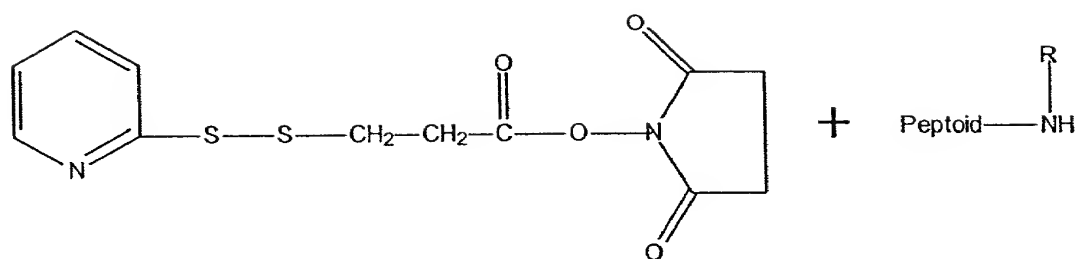


FIGURE 13

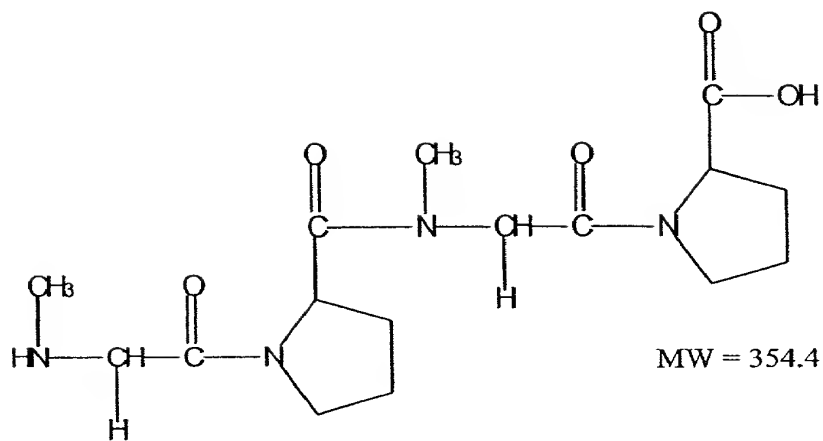


FIGURE 14

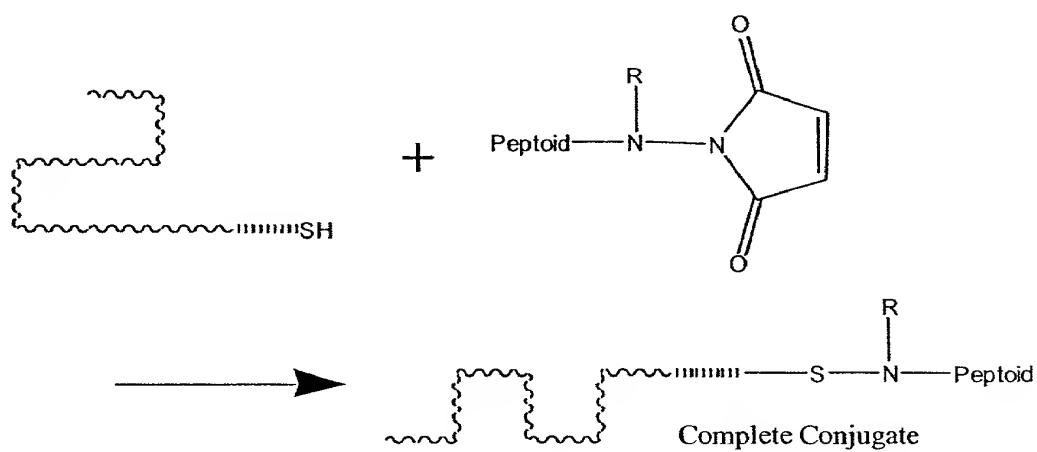
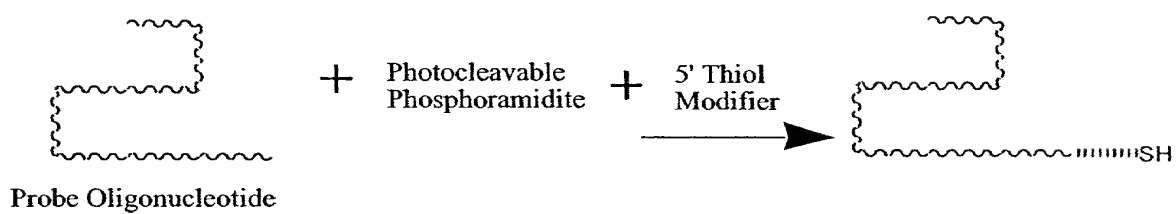
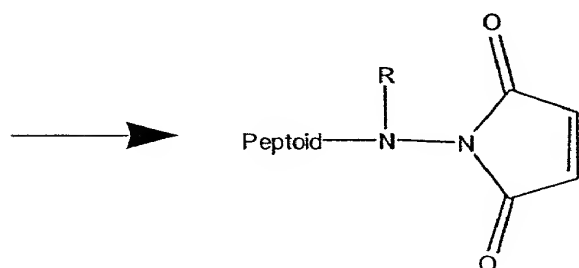
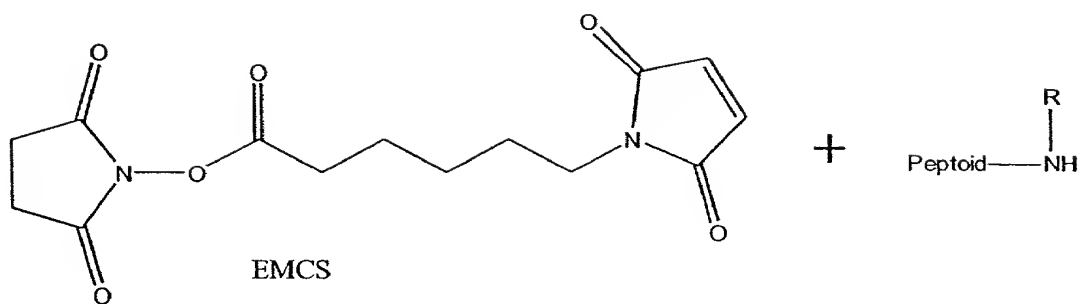
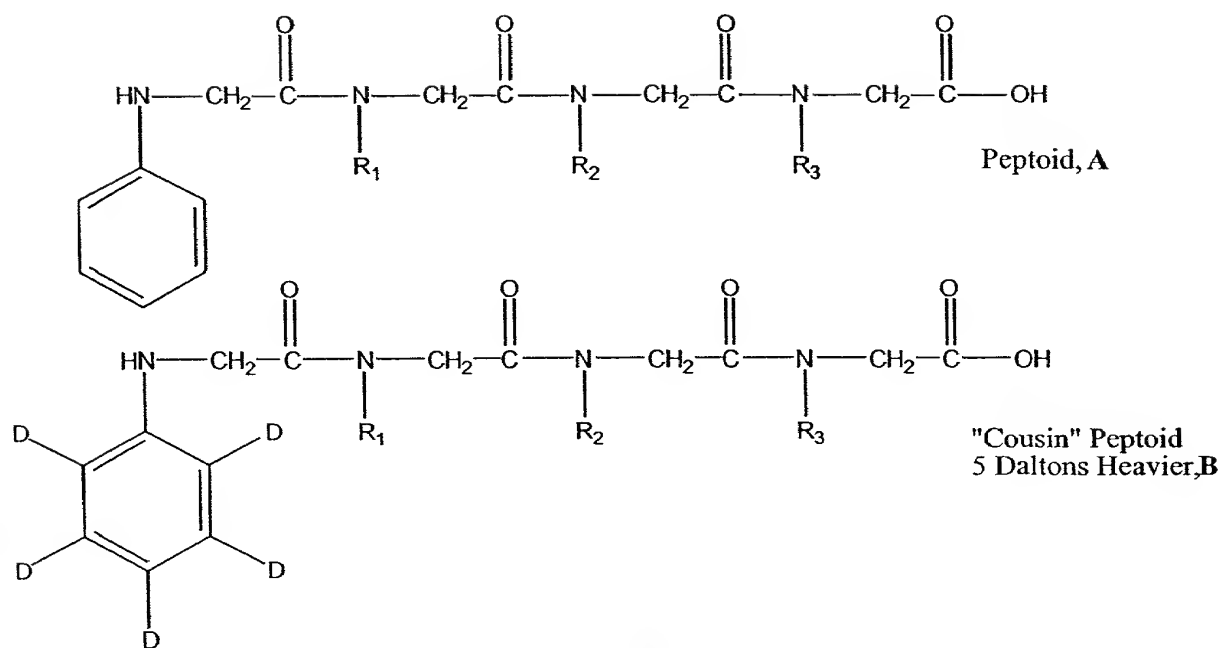


FIGURE 15



Peptoid Library
from mRNA
interrogation
(A-types)

+

Pre-quantitated
"Cousin"
Library
(B-types)



Mass
Spectrometer

Resulting Spectra
Containing Duplexes

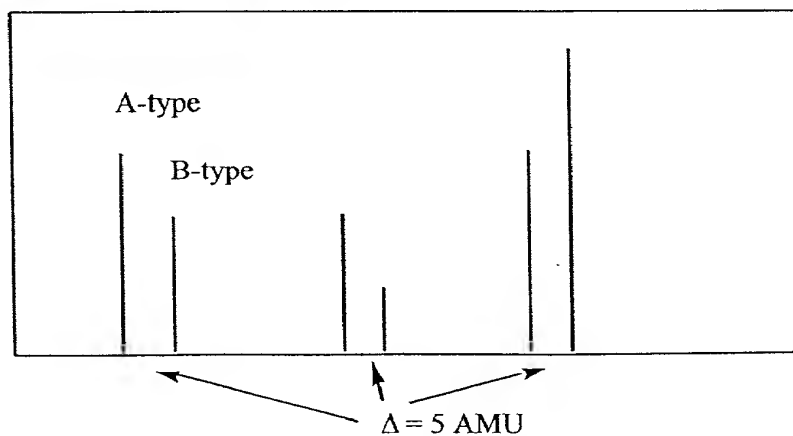


FIGURE 16

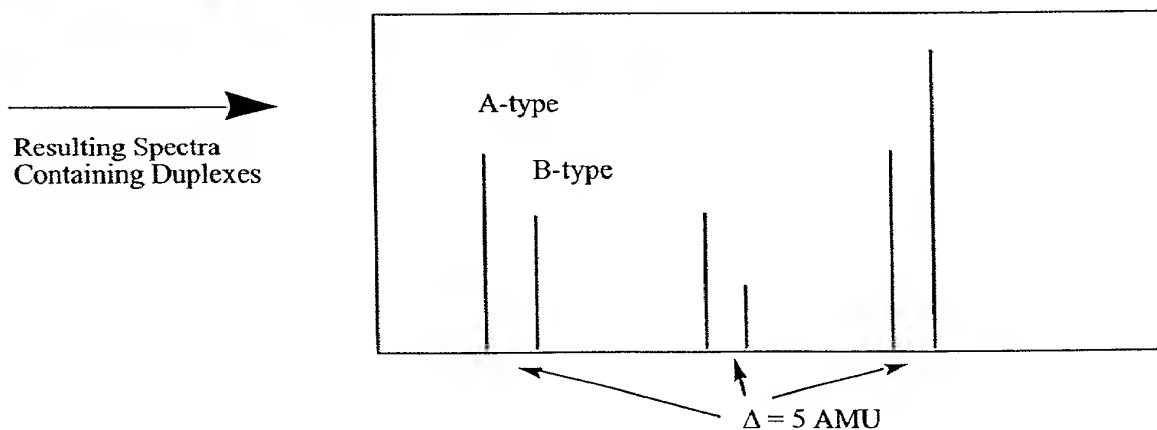
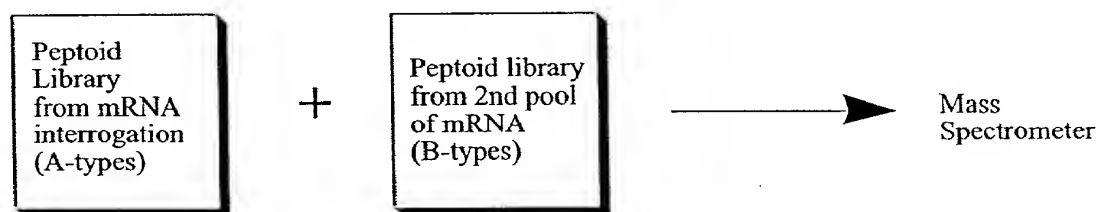
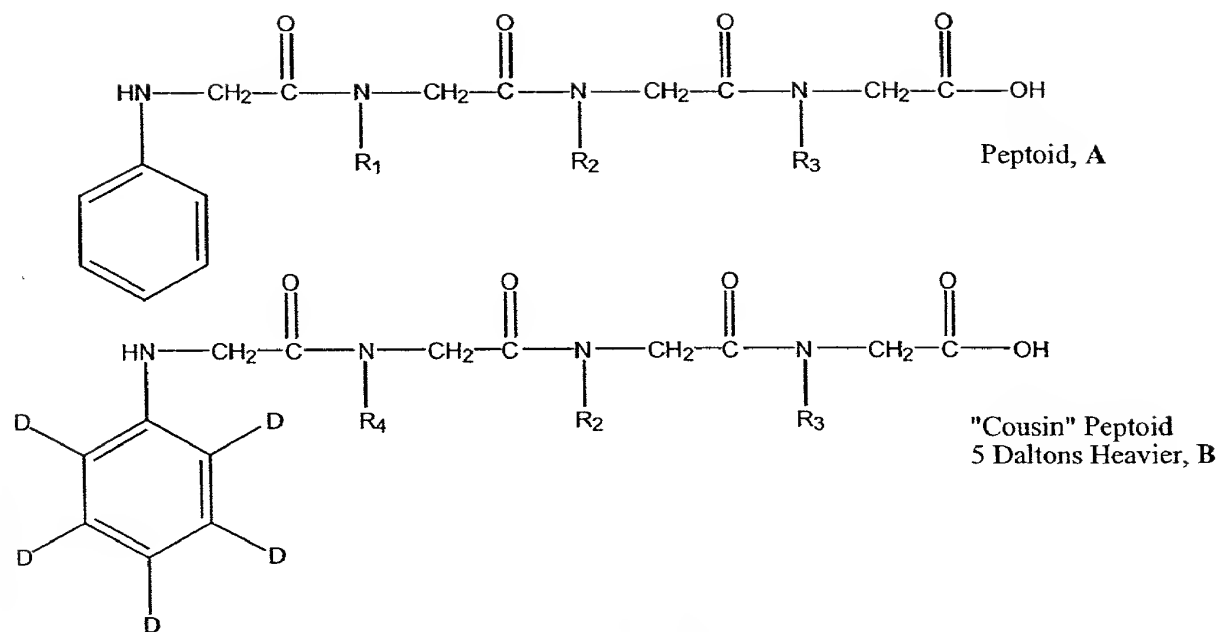
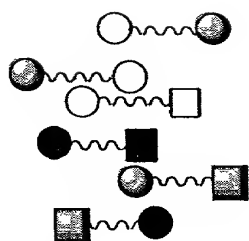


FIGURE 17



Peptoid Library, mixed with isotopic cousins

Rationally designed distribution of masses

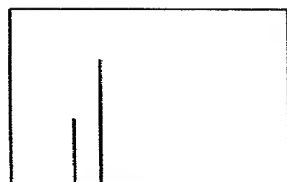
AND

Rationally designed distribution of chromatographic feature



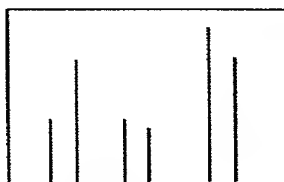
LC, CE, or other chromatography

Mass Spectrometer



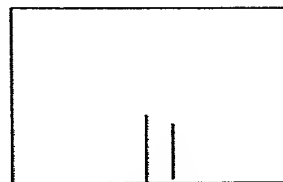
First eluant

Wait...



Later eluant
(several chromatographically similar tags)

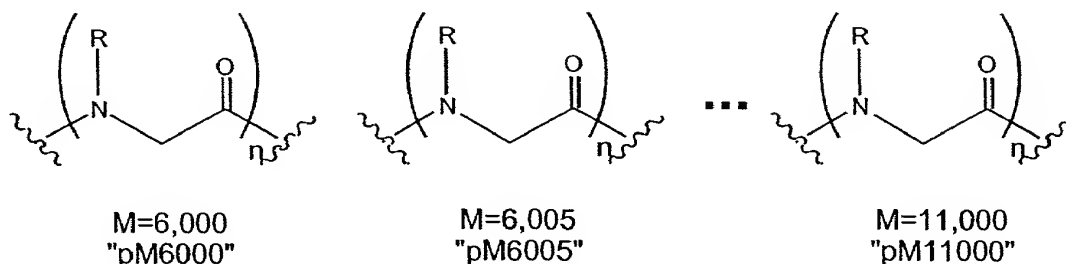
Wait...



Later eluant

FIGURE 18

1) Synthesize and purify 1000 different peptoid oligomer mass tags, of mass 6,000 to 11,000 Daltons.



This will be performed by a robotic synthesizer on solid phase, with oligomer lengths of up to 40 residues. Molecular weights per residue will be 150-300 Daltons.

2) Synthesize and purify 16,000 different DNA oligos, complementary to the mRNA specie to be detected. Create 16 libraries of 1,000 oligos each.

AA(NNNNNNNN)₁, AA(NNNNNNNN)₂, ..., AA(NNNNNNNN)_{1,000}

AC(NNNNNNNN)₁, AC(NNNNNNNN)₂, ..., AC(NNNNNNNN)_{1,000}

.....

TT(NNNNNNNN)₁, TT(NNNNNNNN)₂, ..., TT(NNNNNNNN)_{1,000}

3) Specifically conjugate oligos in each library to a corresponding peptoid mass tag.

AA(NNNNNNNN)₁/pM6000, AA(NNNNNNNN)₂/pM6005, ..., AA(NNNNNNNN)_{1,000}/pM11000

AC(NNNNNNNN)₁/pM6000, AC(NNNNNNNN)₂/pM6005, ..., AC(NNNNNNNN)_{1,000}/pM11000

.....

TT(NNNNNNNN)₁/pM6000, TT(NNNNNNNN)₂/pM6005, ..., TT(NNNNNNNN)_{1,000}/pM11000

4) Purify DNA/peptoid mass tag products and combine library elements into 16 pools.

FIGURE 19

**Mass-tagging on a chip:
How to measure levels of 16,000 mRNA species in solution**

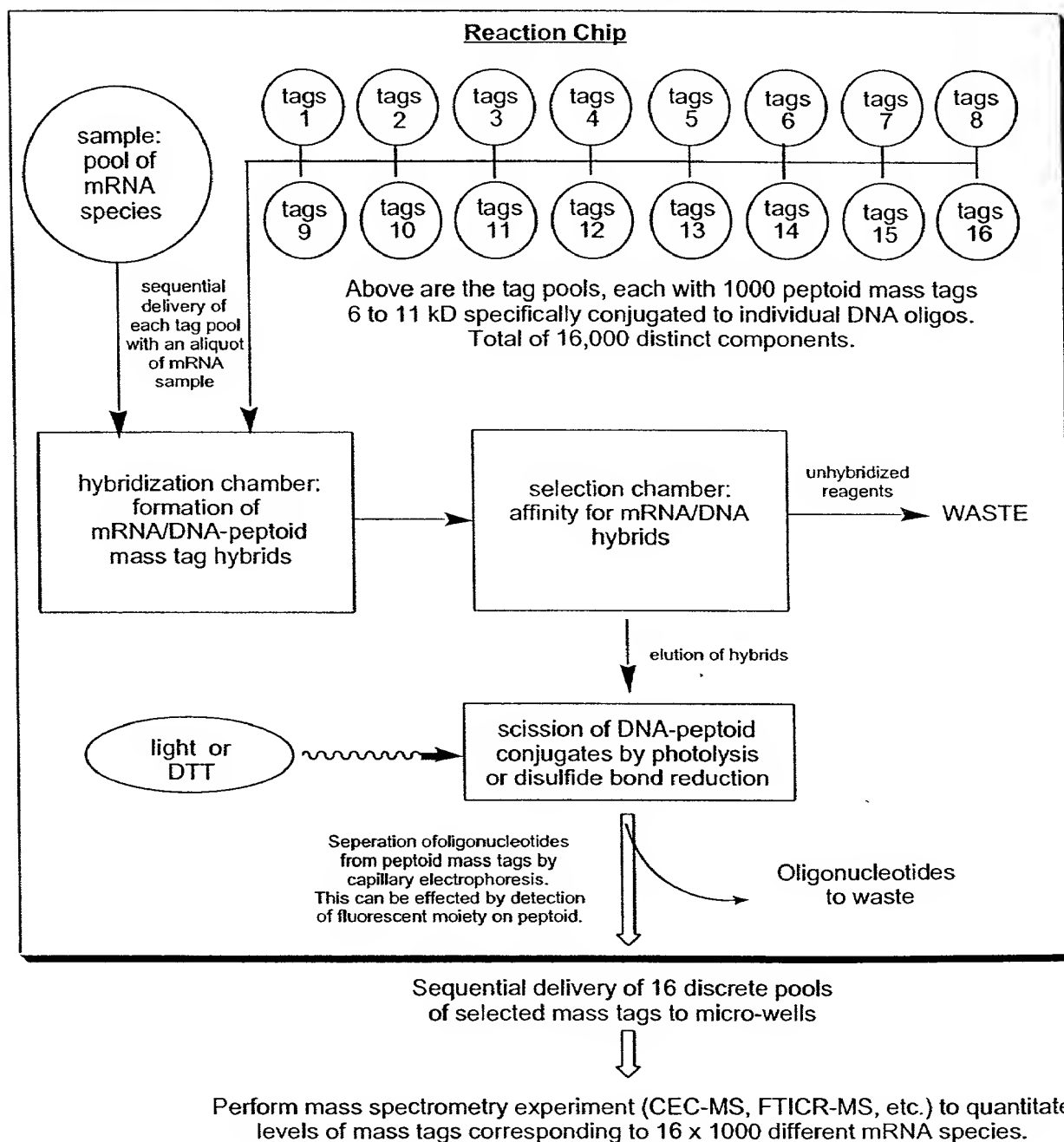


FIGURE 20

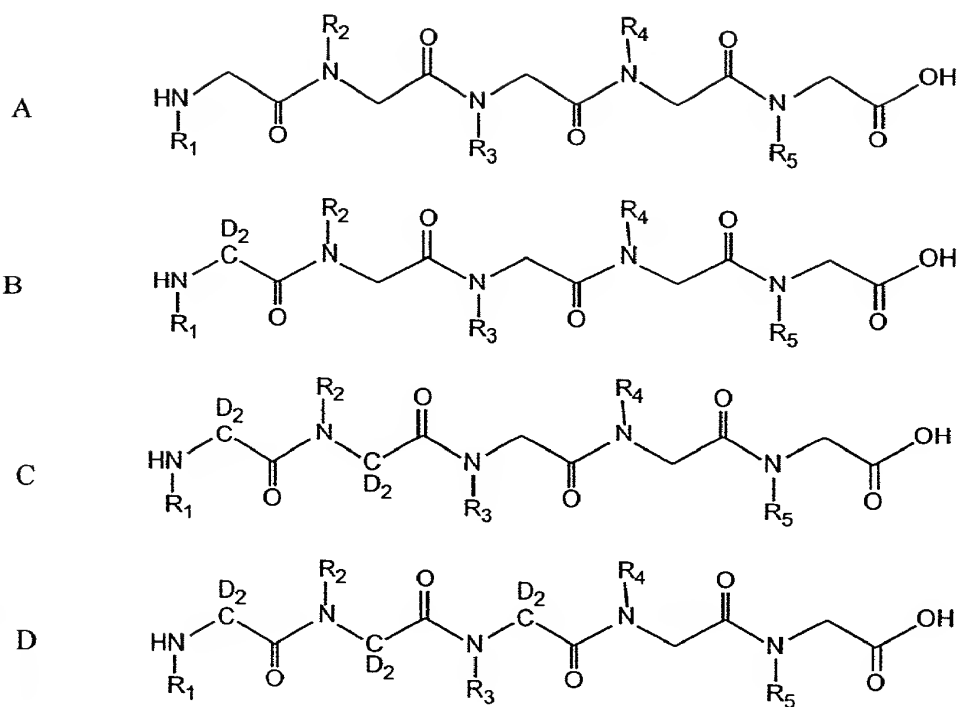


FIGURE 21

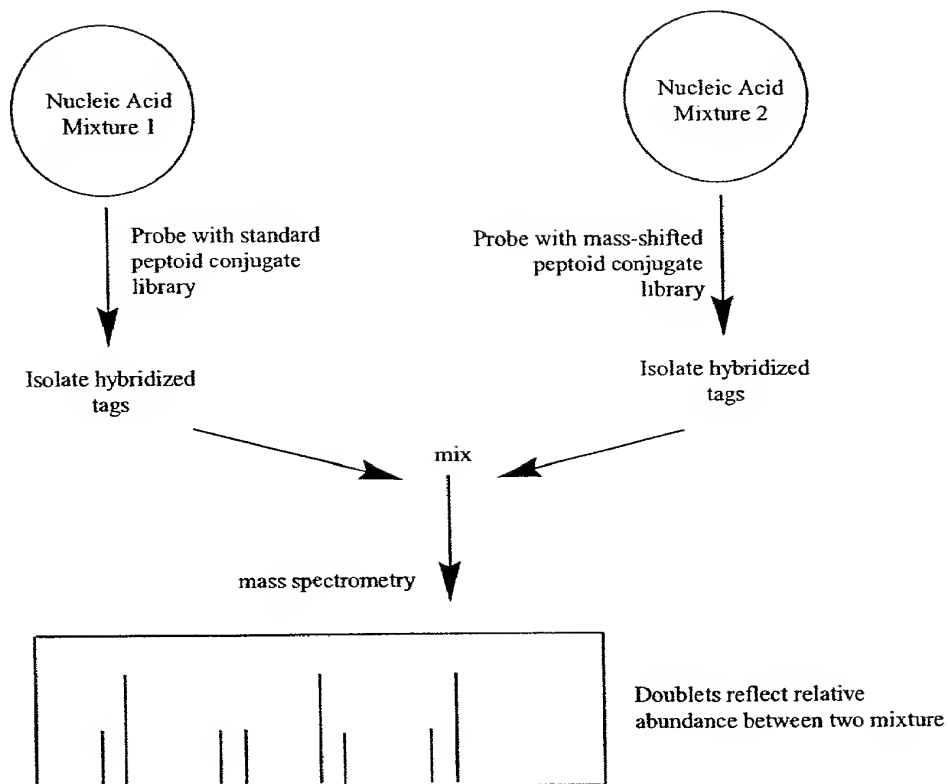


FIGURE 22

10/20/2020 16:59

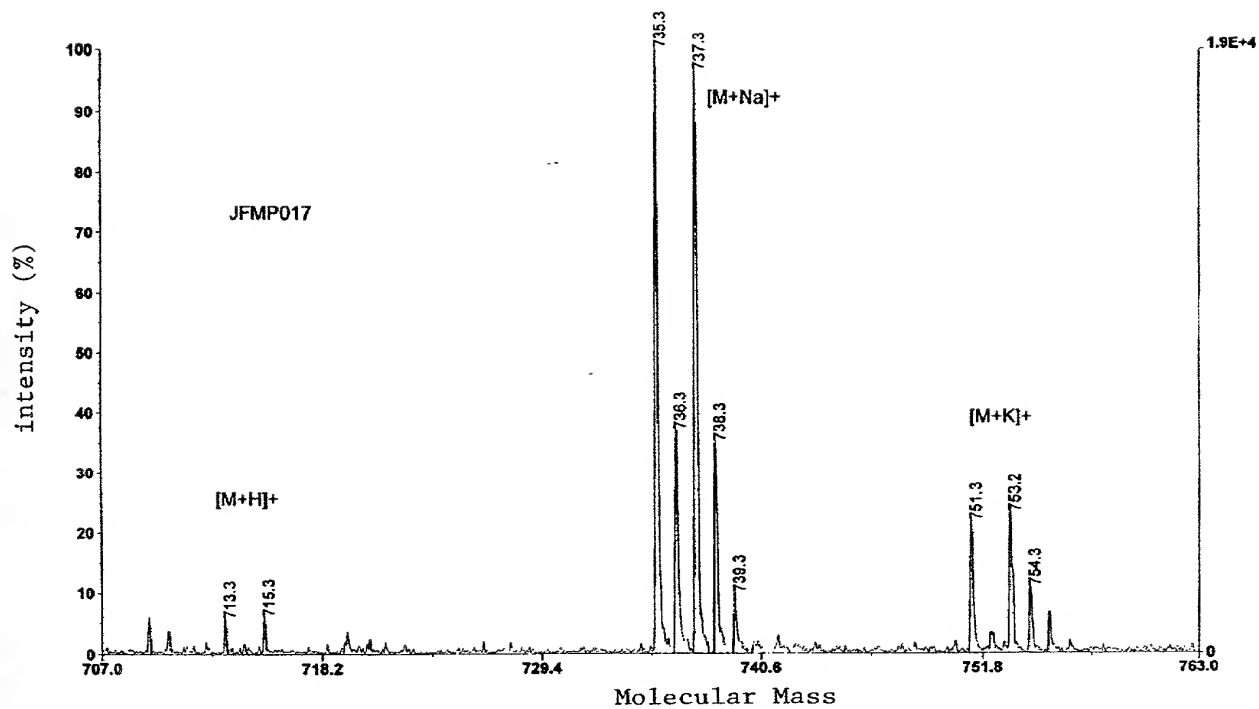


FIGURE 23